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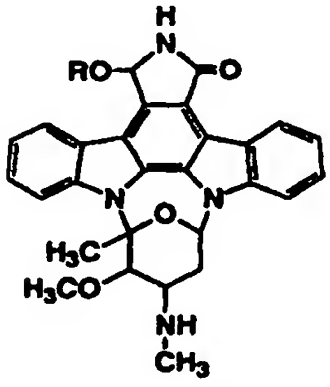
(54) LIPOSOME PREPARATIONS OF INDOLOCARBAZOLE DERIVATIVES

(57) The present invention provides a liposome preparation characterized by encapsulating indolocarbazole derivatives represented by formula (I):



(I)

(wherein R represents hydrogen or lower alkyl) into a liposome comprising lipids.

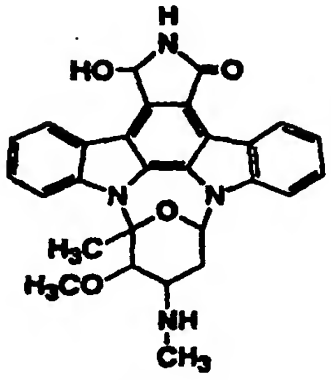
EP 0 850 646 A1

Description

TECHNICAL FIELD

The present invention relates to a liposome preparation containing a medically useful indolocarbazole derivative.

BACKGROUND ART



UCN-01

It is known that UCN-01 has protein kinase C inhibitory activity [J. Antibiotics., 40, 1782 (1987)] and has anti-tumor activity [Cancer Res., 51, 4888 (1991)]. Further, it is disclosed in WO89/07105 that the UCN-01 derivative possesses inhibitory activity on cell growth.

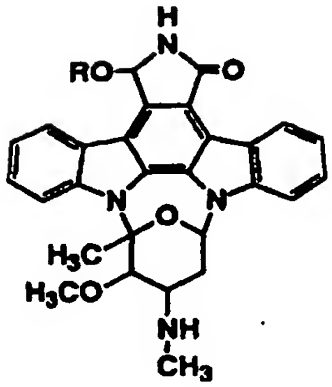
In case UCN-01 or its derivative is administered in vivo particularly to blood vessels, it would be impossible to prevent UCN-01 or its derivative from contacting vascular cells and other various normal cells, as well. Because the UCN-01 derivative has inhibitory activity on cell growth, the contact of UCN-01 or its derivative with normal cells may cause certain adverse effects on normal cells.

In case UCN-01 or its derivative is administered as such to blood vessels, the compound may undergo decomposition in blood or be accumulated in internal organs other than the target, and is thus not necessarily accumulated in tumors effectively.

There is demand for a preparation containing UCN-01 or its derivative, which is stabilized in blood and accumulated at high levels in tumors without causing any effect on normal cells.

DISCLOSURE OF THE INVENTION

The present invention relates to a liposome preparation characterized by encapsulating indolocarbazole derivatives represented by formula (I):



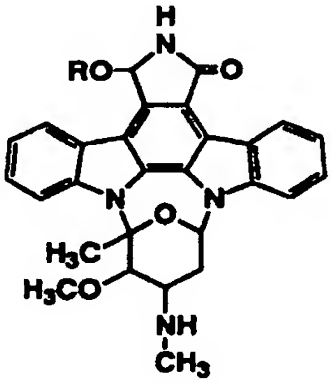
(I)

(wherein R represents hydrogen or lower alkyl) into a liposome comprising lipids.

Hereinafter, the compound represented by formula (I) is referred to as Compound (I).

In the definition for the formula of Compound (I), the lower alkyl refers to straight chain or branched chain of C1 to C6 alkyl, for example, methyl, ethyl, propyl, isopropyl, sec-butyl, tert-butyl, pentyl, hexyl, etc.

The indolocarbazole derivatives represented by formula (I) can be produced by the method described in Japanese Patent Application Laid-Open Publication No. 220,196/87 or WO89/07105. Specific examples of such compounds are shown in Table 1.



(I)

Table 1

Examples of compounds represented by formula (I)	
R	Molecular Weight : MS (m/z)
H	483 $(M+1)^+$
CH_3	497 $(M+1)^+$

Table 1 (continued)

Examples of compounds represented by formula (I)	
R	Molecular Weight : MS (m/z)
C_2H_5	510 $(M)^+$
i-C_3H_7	524 $(M)^+$
n-C_4H_9	538 $(M)^+$

As the lipids for preparation of liposomes, mention is made of phospholipids, glyceroglycolipids, and sphingoglycolipids among which phospholipids are preferably used. Examples of such phospholipids include natural or synthetic phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidic acid, phosphatidylgycerol, phosphatidylinositol, lysophosphatidylcholine, sphingomyelin, egg yolk lecithin and soybean lecithin, as well as hydrogenated phospholipids etc.

The glyceroglycolipids include sulfoxyribosyldiglyceride, diglycosyldiglyceride, digalactosyldiglyceride, galactosyldiglyceride, glycosyldiglyceride, etc.

The sphingoglycolipids include galactosylcerebroside, lactosylserebroside, ganglioside etc. These are used singly or in combination. If necessary, sterols such as cholesterol as membrane stabilizer, tocopherol, etc. as antioxidant, stearylamine, dicetylphosphate, ganglioside, etc. as charged substances, may be used in addition to the lipid component.

Modification of the surfaces of liposomes with a non-ionic surface active agent, cationic surface active agent, anionic surface active agent, polysaccharides and derivatives thereof, polyoxyethylene derivatives etc. can be carried out arbitrarily. Further modification of the surfaces of liposomes with antibodies, proteins, peptides, aliphatic acids etc. can be applied for the purpose of targeting. The solution used for suspending liposomes may be an acid, alkali, various buffers, physiological saline, amino acid infusions etc. in addition to water. Further, antioxidants such as citric acid, ascorbic acid, cysteine, ethylenediaminetetraacetic acid (EDTA) etc. may also be added. Furthermore, preservatives such as paraben, chlorobutanol, benzyl alcohol, propylene glycol etc. may also be added. In addition, glycerin, glucose, sodium chloride etc. can also be added as agents for rendering the solution isotonic.

For production of the liposome preparation of the present invention, a method of preparing a known liposome preparation can be used. The known method of preparing a liposome preparation includes the liposome preparation method of Bangham et al. (J. Mol. Biol., 13, 238 (1965)), the ethanol injection method (J. Cell. Biol., 66, 621 (1975)), the French press method (FEBS Lett., 99, 210 (1979)), the freezing and thawing method (Arch. Biochem. Biophys., 212, 186 (1981)), the reverse phase evaporation method (Proc. Natl. Acad. Sci. (USA), 75, 4194 (1978)), and the pH gradient method (Biochim. Biophys. Acta, 816, 294 (1985); Japanese Patent Application Laid-Open Publication No. 165,560/95).

Among these methods, the pH gradient method has a large number of advantages including, for example, high encapsulation efficiency of Compound (I) in the liposome, the uniform size of the resulting liposomes, a smaller amount of the remaining organic solvent in the liposome suspension. The method of preparing the liposome preparation of the present invention by use of the pH gradient method is as follows: For example, the lipids are dissolved in a solvent such as ether, ethanol etc. and then placed in an round-bottomed flask, and the solvent is evaporated to form a lipid film. Then, an acidic buffer is added to the film, followed by shaking and stirring to form larger multilamellar liposomes. The liposome particles are prepared by the extrusion method etc. so that their average particle diameter is made e.g. about 100 nm. After a weakly acidic solution of Compound (I) is added to this liposome suspension, a suitable buffer is added so that the pH of the liposome suspension is raised at about neutrality (the difference between the pH values of the liposome suspension before and after the rise of pH is preferably 3 or more). By the above operation, Compound (I) can be encapsulated in the inside of the liposomes.

Alternatively, liposomes can also be formed by dissolving Compound (I) and the lipid component in organic solvent such as ethanol etc., then evaporating the solvent off, and adding physiological saline thereto followed by shaking and stirring.

The liposome preparation of the present invention obtained by e.g. the methods described above can be used as such, but can also be lyophilized after adding fillers such as mannitol, lactose, glycine etc. depending on the object of use, storage conditions etc. Lyoprotectants stabilizers such as glycerin etc. may also be added before lyophilization.

The liposome preparation of the present invention is used generally as an injection, but can also be used as an oral dosage form, nasal dosage form, eye drop, percutaneous dosage form, suppository, inhalation etc. by manufacturing the preparation into such forms.

Hereinafter, the Examples and Test Examples of the present invention are shown.

The object of the liposome preparation of the present invention is to stabilize Compound (I) in blood and to increase its accumulation in tumors.

BEST MODE FOR CARRYING OUT THE INVENTION

Example 1

phosphatidylcholine 0.7 g was dissolved in 5 ml ether, and the solvent was evaporated under reduced pressure to form a lipid film. 10 ml of 20 mM citrate buffer, pH 2.5 was added to it and shaken and stirred with a Vortex mixer. Further, this suspension was passed 5 times through 0.4 μm polycarbonate membrane filter. Further, the filtrate was passed 10 times through 0.1 μm polycarbonate membrane filter. A 20 mM citrate buffer, pH 2.5 was added thereto to prepare a liposome suspension containing 50 mg/ml phosphatidylcholine. Separately, 200 mg lactose, 56 mg $Na_2HPO_4 \cdot 12\ H_20$ and 12 mg hydrous citric acid were added to 10 mg UCN-01, and the mixture was dissolved in a distilled water to give 10 ml solution which was then introduced into a glass vial and lyophilized. After lyophilization, the glass vial was returned to normal pressure under a nitrogen stream and sealed to give a lyophilized product of UCN-01 in it. To this lyophilized product was added 2 ml of the previously prepared liposome suspension. Further, 8 ml of 200 mM Na_2HPO_4 solution disodium phosphate was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 2

A liposome suspension was prepared in the same manner as in Example 1 except that the concentration of phosphatidylcholine was made 40 mg/ml by changing the amount of 20 mM citrate buffer, pH 2.5. Separately, 200 mg of lactose, 56 mg of $Na_2HPO_4 \cdot 12\ H_20$, and 12 mg of hydrous citric acid were added to 10 mg UCN-01, and a lyophilized product was prepared in the same manner as in Example 1. To this lyophilized product was added 2 ml of the previously prepared liposome suspension. Further, 8 ml of 200 mM Na_2HPO_4 solution was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 3

A liposome suspension was prepared in the same manner as in Example 1 except that the concentration of phosphatidylcholine was made 30 mg/ml by changing the amount of 20 mM citrate buffer, pH 2.5. Separately, 200 mg of lactose, 56 mg of $Na_2HPO_4 \cdot 12\ H_20$, and 12 mg of hydrous citric acid were added to 10 mg UCN-01, and a lyophilized product was prepared in the same manner as in Example 1. To this lyophilized product was added 2 ml of the previously prepared liposome suspension. Further, 8 ml of 200 mM Na_2HPO_4 solution was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 4

A liposome suspension was prepared in the same manner as in Example 1 except that the concentration of phosphatidylcholine was made 25 mg/ml by changing the amount of 20 mM citrate buffer, pH 2.5. Separately, 200 mg of lactose, 56 mg of $Na_2HPO_4 \cdot 12\ H_20$, and 12 mg of hydrous citric acid were added to 10 mg UCN-01, and a lyophilized product was prepared in the same manner as in Example 1. To this lyophilized product was added 2 ml of the previously prepared liposome suspension. Further, 8 ml of 200 mM Na_2HPO_4 solution was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 5

A liposome suspension was prepared in the same manner as in Example 1 except that the concentration of phosphatidylcholine was made 25 mg/ml by changing the amount of 20 mM citrate buffer, pH 2.5. 5 mg UCN-01 was dissolved by adding 2 ml of the prepared liposome suspension. Further, 3 ml of 200 mM Na_2HPO_4 solution was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 6

A liposome suspension was prepared in the same manner as in Example 1 except that the concentration of phosphatidylcholine was made 20 mg/ml by changing the amount of 20 mM citrate buffer, pH 2.5. 5 mg UCN-01 was dis-

solved by adding 2 ml of the prepared liposome suspension. Further, 3 ml of 200 mM Na_2HPO_4 solution was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 7

A liposome suspension was prepared in the same manner as in Example 1 except that the concentration of phosphatidylcholine was made 15 mg/ml by changing the amount of 20 mM citrate buffer, pH 2.5. 5 mg UCN-01 was dissolved by adding 2 ml of the prepared liposome suspension. Further, 3 ml of 200 mM Na_2HPO_4 solution was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 8

A liposome suspension was prepared in the same manner as in Example 1 except that the concentration of phosphatidylcholine was made 12.5 mg/ml by changing the amount of 20 mM citrate buffer, pH 2.5. 5 mg UCN-01 was dissolved by adding 2 ml of the prepared liposome suspension. Further, 3 ml of 200 mM Na_2HPO_4 solution was added thereto to adjust the pH to 7.4 so that UCN-01 was encapsulated in liposomes.

Example 9

5 mg UCN-01 and 100 mg phosphatidylcholine were dissolved in 15 ml ethanol. The solvent was evaporated under reduced pressure whereby a lipid film was formed. 1 ml of 5 weight-% glucose was added thereto and shaken and stirred with a Vortex mixer. This liposome suspension was passed 4 times through 0.4 µm polycarbonate membrane filter. Further, the filtrate was passed 10 times through 0.1 µm polycarbonate membrane filter so that UCN-01 was encapsulated in liposomes.

Test Example 1

Each of the UCN-01 encapsulating liposomes prepared in Examples 1 to 8 was filtered through 0.45 µm membrane filter to remove insolubles etc. In the case of the liposomes of Examples 1 to 4, 1 ml of 200 mM disodium phosphate-20 mM citrate buffer, pH 7.4 was added there and the mixtures was mixed with 1ml of liposome suspension. These liposomes were ultracentrifuged (110,000 × g, 1 hour) at 10 °C . The phospholipid before and after filtration, and the phospholipid in the supernatant after ultracentrifugation, were quantified by the enzyme method [Practical Clinical Chemistry (enlarged edition), 580 (1982)] using Determiner PL (KYOWA MEDEX CO., LTD.). In addition, UCN-01 before and after filtration, and UCN-01 in the supernatant after ultracentrifugation, were quantified by high performance liquid chromatography. The encapsulation efficiency was calculated by the following formula.

$$\text{Encapsulation efficiency (\%)} = [(A - B)/(C - D)] / (E/F) \times 100$$

A: Concentration of UCN-01 in the filtrate after filtration (mg/ml)
B: Concentration of UCN-01 in the supernatant after ultracentrifugation (mg/ml)
C: Concentration of the phospholipid in the filtrate after filtration (mg/ml)
D: Concentration of the phospholipid in the ultracentrifuged supernatant (mg/ml)
E: Concentration of UCN-01 in the suspension before filtration (mg/ml)
F: Concentration of the phospholipid in the suspension before filtration (mg/ml)

Analytical Conditions for High Performance Liquid Chromatography

Column: Capsule pack PAK C18 UG120 (SHISEID Co., Ltd.) S-5, 4.6 mm × 250 mm
Mobile phase: 20 mM Tris-HCl buffer, pH 9.0 : acetonitrile : tetrahydrofuran = 60 : 22 : 18 (parts by volume).
Flow rate: 0.8 ml/min.
Column temperature: 25 °C.
Detection wavelength: 285 nm.

The results are shown in Table 2.

Table 2

Encapsulation efficiency of UCN-01	
Sample	Encapsulation efficiency of UCN-01 (%)
Example 1	108.3
Example 2	98.1
Example 3	81.8
Example 4	68.4
Example 5	101.6
Example 6	91.8
Example 7	78.7
Example 8	56.8

Test Example 2

To determine the leakage of UCN-01 from liposomes, a UCN-01 encapsulating liposome suspension prepared in the same manner as in Example 1 was introduced into a vial and sealed with a rubber stopper. Obtained samples were stored at different temperatures of 5 °C, 25 °C, and 37 °C, respectively, and the change with time of encapsulation efficiency of UCN-01 was determined. The method of determining the encapsulation efficiency was carried out in the same manner as in Test Example 1.

The results are shown in Table 3.

Table 3

Change with Time of Encapsulation efficiency of Inclusion of UCN-01			
Time	Encapsulation efficiency (%)		
	5 °C	25 °C	37 °C
0	108.2	108.2	108.2
1	93.0	99.3	102.8
3	114.9	96.2	101.5
6	99.2	95.1	100.8
24	93.2	96.5	88.6

As can be seen from Table 2, the liposome preparations of the present invention indicate high encapsulation efficiency of UCN-01. In addition, Table 3 shows that the liposome preparations of the present invention are stable liposome preparations with less leakage of UCN-01.

Example 10

1 g phosphatidylcholine was dissolved in 5 ml ether, and the solvent was evaporated under reduced pressure to form a lipid film. 10 ml of 20 mM citrate buffer, pH 4.0 was added thereto and shaken and stirred with a Vortex mixer. Further, this suspension was passed 5 times through 0.4 µm polycarbonate membrane filter. Further, the filtrate was passed 10 times through 0.1 µm polycarbonate membrane filter. A 20 mM citrate buffer, pH 4.0 was added thereto to prepare a liposome suspension containing 50 mg/ml phosphatidylcholine. Separately, 200 mg lactose, 56 mg $Na_2HPO_4 \cdot 12\ H_2O$ and 12 mg hydrous citric acid were added to 10 mg UCN-01, and its lyophilized product was prepared in the same manner as in Example 1. To this lyophilized product was added 2 ml of the previously prepared lipo-

some suspension. Further, 8 ml of 28.2 mM aqueous sodium hydroxide was added thereto to adjust the pH to 8.0 so that UCN-01 was included in liposomes.

Example 11

UCN-01 was encapsulated in liposomes in the same manner as in Example 10 except that 1.2 g phosphatidylcholine and 0.3 g cholesterol were used as the starting materials of the lipid film.

Example 12

UCN-01 was encapsulated in liposomes in the same manner as in Example 10 except that 1.2 g phosphatidylcholine, 0.4 g cholesterol and 0.4 g PEG-DSPE (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[poly-(ethyleneglycol) 2000]; a product of AVANTI POLAR LIPIDS INCORPORATION)) were used as the starting materials of the lipid film.

Example 13

A liposome suspension was prepared in the same manner as in Example 10 except that the concentration of phosphatidylcholine in the liposome suspension was made 35 mg/ml by changing the amount of 20 mM citrate buffer, pH 4.0. Separately, a lyophilized product of UCN-01 was prepared in the same manner as in Example 10. To this lyophilized product was added the previously prepared liposome suspension so that UCN-01 was made 0.5 mg/ml. A 8 ml of 10.4 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 14

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 13. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.05 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 15

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 13. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.005 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 16

UCN-01 was encapsulated in liposomes in the same manner as in Example 13 except that 0.2 μm polycarbonate membrane filter was used in place of the 0.1 μm polycarbonate membrane filter to prepare a liposome suspension.

Example 17

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 16. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.05 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 18

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 16. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.005 mg/ml. A 8 ml of 9.0 mM aqueous sodium was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 19

0.9 g phosphatidylcholine and 0.1 g phosphatidylethanolamine were evaporated in 5 ml chloroform, and the solvent was distilled off under reduced pressure whereby a lipid film was formed. 10 ml of 20 mM citrate buffer, pH 4.0 was added thereto and shaken and stirred with a Vortex mixer. This suspension was passed 5 times through 0.4 μm polycarbonate membrane filter. Further, the filtrate was passed 10 times through 0.1 μm polycarbonate membrane filter. 20 mM citrate buffer, pH 4.0 was added thereto to prepare a liposome suspension containing 45 mg/ml phosphatidylcholine. Separately, a lyophilized product of UCN-01 was prepared in the same manner as in Example 10. To this lyophilized product was added the previously prepared liposome suspension so that UCN-01 was made 0.5 mg/ml. A 8 ml of 10.4 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 20

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 19. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.05 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 21

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 19. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.005 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 22

0.7 g phosphatidylcholine and 0.3 g phosphatidylgycerol were dissolved in 5 ml chloroform, and the solvent was evaporated under reduced pressure whereby a lipid film was formed. 10 ml of 20 mM citrate buffer, pH 4.0 was added thereto and shaken and stirred with a Vortex mixer. This suspension was passed 5 times through 0.4 μm polycarbonate membrane filter. Further, the filtrate was passed 10 times through 0.1 μm polycarbonate membrane filter. A 20 mM citrate buffer, pH 4.0 was added thereto to prepare a liposome suspension containing 35 mg/ml phosphatidylcholine. Separately, a lyophilized product of UCN-01 was prepared in the same manner as in Example 10. To this lyophilized product was added the previously prepared liposome suspension so that UCN-01 was made 0.5 mg/ml. 8 ml of 10.4 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 23

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 22. The liposome suspension was added to this lyophilized product such that UCN-01 was made 0.05 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 24

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 22. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.005 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 25

UCN-01 was encapsulated in liposomes in the same manner as in Example 22 except that 0.7 g phosphatidylcholine and 0.3 g cholesterol were used as the starting materials of the lipid film.

Example 26

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 25. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.05 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Example 27

A liposome suspension and a lyophilized product of UCN-01 were prepared in the same manner as in Example 25. The liposome suspension was added to this lyophilized product so that UCN-01 was made 0.005 mg/ml. A 8 ml of 9.0 mM aqueous sodium hydroxide was added to 2 ml of this solution to adjust the pH to 8.0 so that UCN-01 was encapsulated in liposomes.

Test Example 3

The UCN-01 encapsulating liposomes prepared in Examples 10 to 12 were treated in the same manner as in Test Example 1 and the encapsulation efficiency of UCN-01 in each liposome was examined. The results are shown in Table 4.

Table 4

Encapsulation efficiency of UCN-01 (%)	
Sample	Encapsulation efficiency of UCN-01 (%)
Example 10	100.0
Example 11	85.7
Example 12	82.9

Test Example 4

The UCN-01 encapsulating liposomes prepared in Examples 13 to 27 were ultracentrifuged (110,000 × g, 2 hours) at 10 °C. UCN-01 before ultracentrifugation, and UCN-01 in the supernatant after ultracentrifugation, were quantified by high performance liquid chromatography. The encapsulation efficiency was calculated by the following formula:

$$\text{Encapsulation efficiency of UCN-01 (\%)} = (B - A) \times 100/B$$

A: Concentration of UCN-01 in the ultracentrifuged supernatant (mg/ml)
B: Concentration of UCN-01 in the suspension before ultracentrifugation (mg/ml)

Analytical Conditions for High Performance Liquid Chromatography

Column: YMC AM-312, 6.00 mm diameter × 150 mm length (manufactured by YMC Co., Ltd.).
Mobile phase: 0.05 M phosphate buffer (plus 0.1 % triethylamine), pH 7.3 : acetonitrile = 1 : 1 (part by volume).
Flow rate: 1.0 ml/min.
Column temperature: 25 °C.
Detection: Excitation wavelength 310 nm, Emission wavelength 410 nm.

The results are shown in Table 5.

Table 5

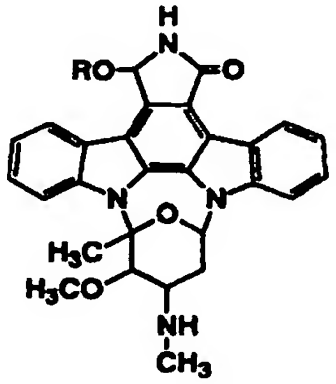
Encapsulation efficiency of UCN-01	
Sample	Encapsulation efficiency of UCN-01 (%)
Example 13	98.7
Example 14	99.0
Example 15	96.8
Example 16	99.2
Example 17	99.2
Example 18	94.0
Example 19	99.9
Example 20	99.7
Example 21	88.5
Example 22	98.9
Example 23	99.4
Example 24	100.0
Example 25	99.6
Example 26	99.4
Example 27	92.0

INDUSTRIAL APPLICABILITY

According to the present invention, there is provided a liposome preparation in which a medically useful indolocarbazole derivative has been included.

Claims

1. A liposome preparation characterized by encapsulating indolocarbazole derivatives represented by formula (I):



(I)

(wherein R represents hydrogen or lower alkyl) into a liposome comprising lipids.

2. A liposome preparation according to claim 1 wherein lipids is phospholipids.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP97/02033

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl^6 A61K31/55, C07D491/22, A61K9/127
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int. Cl^6 A61K31/55, C07D491/22, A61K9/127

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO, 89/07105, A (Kyowa Hakko Kogyo Co., Ltd.), August 10, 1989 (10. 08. 89) & EP, 383919, A	1, 2
Y	JP, 7-278016, A (Pola Chemical Industries Inc.), October 24, 1995 (24. 10. 95)(Family: none)	1, 2
Y	JP, 3-163031, A (NOF Corp.), July 15, 1991 (15. 07. 91)(Family: none)	1, 2

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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